BRIEF COMMUNICATION



The first observation of copulation in Andean catfish Astroblepus ubidiai (Siluriformes, Astroblepidae), in Lago San Pablo, Imbabura, Ecuador

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Abstract

The reproductive behaviour of the Andean catfish *Astroblepus ubidiai* was observed directly and with an underwater camera in a spring located next to Lago San Pablo in the province of Imbabura, Ecuador, in late 2015 and early 2016. Five mating attempts were observed, four of which were video-taped, and two of which resulted in complete copulation. During copulation, the male grabs the female from the top of the head with its buccal disc and pelvic fins and, from this position, bends its body forming a "C" to reach the genital pore of the female and introduce its copulatory organ. Once copulation is complete, the female swims away rapidly. Details of the copulation are described here through illustrations.

KEYWORDS

copulation, courtship, fish, freshwater, reproduction

1 | INTRODUCTION

Astroblepid catfish are climbing fish with fleshy buccal discs and thickened and highly mobile pelvic fins that allow them to scale high-flow, steep-sloped streams (Maldonado-Ocampo et al., 2005). They are typically small fish less than 15 cm in length, although some species can reach up to 30 cm in length (Burgess, 1989). Astroblepids are restricted to Andean Mountain streams from Panama to Bolivia and occur at elevations between 100 and 4600 m, although they are most common between 500 and 2000 m (Ardilla, 2015; Buitrago-Suárez, 1995; Roman-Valencia, 2001; Schaefer & Buitrago, 2002; Schaefer et al., 2011, Schafer & Arroyave, 2010). Most Astroblepids have restricted distributions and are limited to small areas at elevations above 1000 m, so endemism is common (Buitrago & Galvis, 1997; Burgess, 2004; Collette, 1962). There are 58 species of Astroblepids (Van der Sleen & Albert, 2018), 24 of which occur in Ecuador (Barriga, 2012), inhabiting subtropical, temperate and high Andean ecosystems on both sides of the Andes (Jimenez-Prado, 2010). Astroblepids generally inhabit clear waters and are sensitive to environmental degradation, so they are good indicators of the health

status of rivers (Schaefer *et al.*, 2011). They are especially sensitive to the presence of invasive species such as the rainbow trout (*Oncorhynchus mykiss*), which feed on them (Torrijos *et al.*, 2016).

The Andean catfish (Astroblepus ubidiai), known locally as "preñadilla" or "challua," is a multivoltine species, endemic to the province of Imbabura in Ecuador (Pellegrini, 1931; Vélez-Espino, 2003, 2004), and is considered critically endangered (Arguello & Jiménez-Prado, 2016). It inhabits the upper basin of the Mira River, where it was abundant in streams, ditches and springs (Mena-Valenzuela & Valdiviezo-Rivera, 2016; Vélez-Espino, 2004). Nonetheless, it has decreased drastically in abundance and is now relatively rare due to the fragmentation, modification and contamination of their habitat, as well as the introduction of goldfish (Carassius auratus) and largemouth bass (Micropterus salmoides) into Lago San Pablo (Vélez-Espino, 2004). Currently, A. ubidiai inhabits springs and streams around Lago San Pablo and is also present in the streams that are born to the north and the south of the Mojanda nudo, in the Ambi River and its tributary streams (Vélez-Espino, 2004; Vélez-Espino & Rueda, 2004; Obs per. P. Mena-Valenzuela). Like most troglophilic fish, A. ubidiai reproduces in groundwater (Brown & Johnson, 2001; Trajano, 2001), and gravid

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females have been observed throughout the year (Vélez-Espino, 2004). A. *ubidiai* is sexually dimorphic, with males having an external copulatory organ and females having a genital pore, suggesting that insemination occurs in this species (Vélez-Espino, 2004). Insemination had previously been suggested by Burgess (1989), because of the presence of an elongated "urogenital" papilla in males that would function as an organ for insemination and has been reported for other Astroblepids including *Astroblepus chotae*, *Astroblepus sabalo* and *Astroblepus trisfasciatus* based on the presence of spermatozoa in the ovaries (Javonillo *et al.*, 2009; Spadella *et al.*, 2012). Unfortunately, direct observations of insemination have not been published, and there have been no studies on the reproductive behaviour of *A. ubidiai*, despite efforts made during previous ecological studies of this species (Vélez-Espino, 2004).

In this study, the authors examined the reproductive behaviour of A. *ubidiai* in a spring close to Lago San Pablo, making daytime observations that were recorded and filmed in late 2015 and early 2016. The reproductive behaviour of A. *ubidiai*, including the observation of copulation, is described for the first time, and various positions adopted during mating are illustrated.

2 | STUDY AREA

The study took place at the "Sumak Yaku" or "Proaño" Spring, located on the southeastern side of Lago San Pablo (0° 12′ 31.31′′ N-78° 12´ 23.23´´ W), at 2685 m of altitude and 208 m from the shore of the lake, in the Arague parish of Cantón Otavalo, Imbabura Province. Ecologically, the area corresponds to the flooded lacustrine montane grassland of the Andes (Ministerio del Ambiente del Ecuador (MAE), 2013). The spring is enclosed with concrete walls, and the water is poured through a tube into two pools built with brick and concrete. The first pool is covered by a roof that does not allow direct entry of sunlight and prevents the growth of plants and algae, whereas the second pool is open, which favours the growth of aquatic plants, algae and aquatic invertebrates, and has a surface area of 180 m² and a depth of 90 cm. The walls and the bottom of the pool are cracked by erosion, allowing water to pass and forming cavities that serve as a refuge for A. ubidiai. The water in the pools is transparent, and point measures taken during sampling gave a water temperature 16.5°C, pH 6.5, dissolved oxygen 13.33 mg I^{-1} , conductivity 258 μ S I^{-1} and total salts 124 mg l⁻¹. Water from the pools is pumped into large tanks located 360 m away, where it is treated with chlorine to provide potable water to about 3000 users in the surrounding areas, whereas the rest flows to Lago San Pablo through a small stream. The water flows permanently generating a flow rate of 82 $I s^{-1}$.

3 | MATERIALS AND METHODS

Underwater observations were conducted during daylight hours in 2015 and 2016 through visual observation and using a GOPRO HERO3 video camera, as has been performed routinely in fish ecology

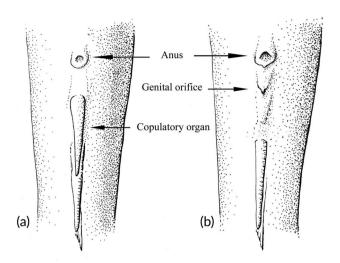


FIGURE 1 Differences between male (a) and female (b) *Astroblepus ubidiai* external sex organs; museum specimens MECN-DP 3387

and ethology studies (Coad, 1993; Dollof et al., 1996; Helfman, 1983; Nakamura et al., 2004; Sabino, 1999). The observations were conducted every 8 days from 03.30 to 05.30 hours from 14 June 2015 to 6 February 2016. The time of day was chosen because observations made prior to the start of the study indicated that this was a period during which A. ubidiai exhibited great activity. In total, the spring was visited 21 times for a total of 42 h of observation, and approximately, 20 min of these observations was filmed during each visit, for a total of 420 min of video recordings. The camera was placed in front of small crevices or caverns located at the base of the walls of the pool. To estimate the size of the A. ubidiai population in the spring, the individuals present were counted during each visit in the evening, when fish were most active. The length of the male copulatory organ was estimated from measures of 12 preserved specimens in the collection of the Instituto Nacional de Biodiversidad (INABIO) in Quito (catalogue number: MECN-DP 3387-3388).

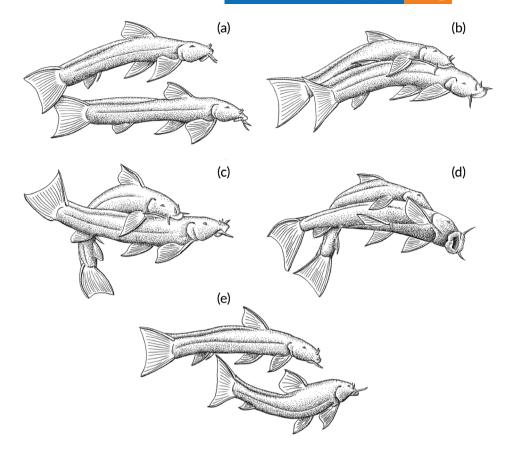
4 | ETHICAL STATEMENT

This research complied with the requirements established in the Environmental Unified-Text-of-Secondary-Legislation of (Texto Unificado de Legislación Secundaria de Medio Ambiente-TULSMA) regarding animal welfare laws, guidelines and policies approved by the Ministry of Environment, Water and Ecological Transition (Ministerio de Ambiente, Agua y Transición Ecológica del Ecuador-MAATE), through the Authorization of Scientific Research No. 05-2016-IC-FAU-FLO-DPAI/MAE.

5 | RESULTS

Averaging across visits, approximately 120 specimens of A. ubidiai inhabit the open pool in the Sumak Yaku or Proaño Spring. The

FIGURE 2 Mating sequence of Astroblepus ubidiai. The positions of the male and female are shown, from left to right. (a) The male positions himself parallel to the female. (b) The male comes to the left side of the female. (c) The male holds the female with the buccal disc and fins and then bends his body in the shape of a "C" with his copulatory organ erect. (d) The male introduces the copulatory organ into the female's genital pore. (e) The female swims away



transparency of the water made it easy to see the fish and the caverns and crevices at the bottom of the pool where the fish tended to concentrate when not searching for food. Individuals frequently grouped together in one of the caves, which is where copulation was observed.

Like all Astroblepids, this species presented clearly differentiated sex organs. Females have a genital orifice, and males have a copulatory organ that measured 7.95 mm on average (n=12, S. D. =1.01 mm; Figure 1).

Reproductive behaviour was observed on five occasions: three of these were attempts at copulation, and two included full copulation. In the first three cases, the pair was observed leaving a small cave, with the male quickly approaching the female and trying to hold her with his pectoral fins from above, but the female freed herself by shaking her body and rapidly swam away.

The first mating with copulation was recorded at 16.20 hours on 5 December 2015 and lasted 5 s, whereas the second occurred on 6 February 2016 at 15.33 hours and lasted 8 s. The fish involved measured approximately between 9 and 10 cm in length. During mating, the male held the female by fixing his mouth on the dorsal side of the female's body anterior to the dorsal fin and used his pectoral fins to hold the sides of her dorso near the middle of the body (Figure 2a,b). Then, the male arched his body forward and adopted a "C-shaped" curve until his copulatory organ briefly came into contact with the female's genital orifice (Figure 2c,d). Finally, the male released the female, and she swam quickly away (Figure 2e).

No courtship behaviour was seen before each copulation event; the males would catch the females off guard, and then a struggle began in which the female tried to escape while the male tried to copulate. There was also no indication that males compete with one another for access to females, and males are similar in size to females, so neither sex seems to have a body size advantage during the mating struggles.

6 | DISCUSSION

The reproductive strategies of fish are varied and striking. Fish commonly present courtships involving unique body movements, as well as male nuptial colourations that are sexually selected by females (Brooks & Endler, 2001; Hamilton & Poulin, 1999; McFarland, 1999). For example, male guppies (Poecilia reticulata) exhibit special body and fin movements during courtship that females use as a basis for mate selection (Brown & Harper, 1993; Kodric-Brown & Nicoletto, 2001). In other cases, the males compete through ritualized combat, in which they display their abilities and characteristics like body size and colour to impress females (Iwaniuk, 2001; O'Rian & Jarvis, 1997). Other species display complex sexual behaviours and ornaments (Brooks & Endler, 2001; McFarland, 1999), like male Slopehead catfish (Ageneiosus magoi), which develop sexual dimorphism seasonally in the form of modifications of the maxillary barbels, the dorsal fin and the anterior region of the anal fin, the latter harbouring a copulatory organ (Castillo & Brull, 1989). Andean catfish (A. ubidiai) did not exhibit any

complex mating behaviours, modifications of barbels or fins or sexual dimorphism other than in their sexual organs.

Descriptions of mating behaviour in *Astroblepus* are relatively scarce. Buitrago and Galvis (1997) observed the reproductive behaviour of *Astroblepus micrescens* in aquaria. They showed that the males approached the females to make rapid contact with their genital region but did not introduce the copulatory organ or "urogenital" papilla into the females' urogenital pores. This behaviour is similar to three of the reproductive encounters documented here for *A. ubidiai*, in which copulation did not occur.

Nonetheless, the authors observed full copulation, that is, the introduction of the male organ into the female's orifice, twice in this study, confirming insemination in A. *ubidiai*, as documented previously for the genus based on the presence of spermatozoa in the ovaries of female A. *sabalo*, A. *chotae* and A. *trifaciatus* (Javonillo *et al.*, 2009; Spadella *et al.*, 2012). Given that the presence of copulatory organs is widespread in male Astroblepus (Burgess, 1989), insemination probably occurs in other species of the family Astroblepidae. This is noteworthy because the only other catfish families in which insemination is known are the Scoloplacidae and the Auchenipteridae (Spadella *et al.*, 2012).

One caveat is that Andean catfish (A. ubidiai) in the Sumak Yaku Spring were observed during the evening. It is possible that mating behaviour differs at night or at other times of the day, and there is male competition for access to females or courting, as happens in other fish groups. Future studies should examine the mating behaviour of this species at other times, including at night. The observations were also made at a single site, so conducting studies at other sites is also recommended as it is possible that mating behaviours differ among sites. Finally, mating behaviour may differ substantially among species of Astroblepus, so studies conducted on other species are also necessary to obtain a better understanding of the mating behaviours of this important and highly specialized catfish family.

Knowledge of habitat requirements during the reproductive season reveals the importance of small caverns as refuges and places for social interactions for A. *ubidiai* and highlights the need to conserve healthy aquatic ecosystems exhibiting the structural complexity needed by this species to ensure its survival.

This study represents the first published observation of the reproductive behaviour of Andean catfish (A. *ubidiai*) in a natural environment and documents the occurrence of copulation in this species.

AUTHOR CONTRIBUTIONS

P.M.-V. contributed in research in correction, idea, sampling organization, writing, correction, editing and improvement of the manuscript. J.V.-R. helped in lab work, and partly writing, correcting, editing and improving the manuscript. J.M.-O. helped in field work and partly writing and correcting the manuscript. W.E.A. contributed in partly writing, reviewing English language, editing and improving the manuscript.

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